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Sero-prevalence and Associated Risk Factors of Hepatitis B Virus and Hepatitis C Virus Infections Among Pregnant Women at Dessie Referral Hospital, Northeast Ethiopia.

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A thesis submitted to the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar for the partial fulfillment of the requirements for the degree of Master of Sciences in Infectious and Tropical Diseases.

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CERTIFICATE

This is to certify that the thesis entitled "Sero-prevalence and Associated Risk Factors of Hepatitis B Virus and Hepatitis C Virus Infections among Pregnant Women at Dessie Referral Hospital, Northeast Ethiopia" submitted by Mr. Mohammed Seid for the award of MSc. Degree in Infectious and Tropical Diseases was carried out under our supervision and the thesis has not been previously submitted in part or full for any degree or diploma of this or any other University.

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DECLARATION

The research work in this thesis entitled “Sero-prevalence and Associated Risk Factors of Hepatitis B Virus and Hepatitis C Virus Infections among Pregnant Women at Dessie Referral Hospital, Northeast Ethiopia” was carried out by me under the supervision of Dr. Baye Gelaw and Mr. Abate Assefa in the College of Medicine and Health Sciences, School of Biomedical and Laboratory Sciences, for the award of MSc Degree in Infectious and Tropical Diseases. I declare that this work is original and has not been submitted to any other University or institution.

Name of student: Mohammed Seid

Place: Gondar

Date: 13/06/2014

Signature: _____

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List of Abbreviations

ANC	Antenatal Care Clinic
CD	Cluster of Differentiation
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
HBeAg	Hepatitis B “e” Antigen
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
Ml	Milliliter
RNA	Ribonucleic Acid
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization

Abstract

Background: Hepatitis B and hepatitis C viruses are the most common types of viruses that infect the liver. Infection by these viruses during pregnancy have high rate of vertical transmission and have adverse effect on both the mother and child. Infection at the prenatal age and infancy usually leads to a chronic carrier status. Epidemiological data for these viruses are important to take appropriate preventive measures.

Objective: The aim of this study was to determine the sero-prevalence and associated risk factors of hepatitis B virus and hepatitis C virus infections among pregnant women at Dessie referral hospital.

Methods: A cross sectional study was conducted from March to May, 2014 at Dessie referral hospital antenatal care clinic. A pre-tested structured questionnaire was used to collect data on socio-demographic and other variables. Five milliliter of venous blood was collected from each study subject. Serum was separated and tested for hepatitis B surface antigen (HBsAg) and anti-HCV antibody using rapid test kits. Data was entered and analyzed using SPSS version 16.0 computer software. Logistic regression analysis was employed to examine the possible risk factors of hepatitis B virus and hepatitis C virus infections. P-value < 0.05 was considered as statistically significant.

Results: A total of 385 pregnant women were involved in this study and their mean age was 28.16 ± 5.37 years. The overall prevalence of HBsAg and anti- hepatitis C virus antibody was 4.9% and 0.8% respectively. High proportion of HBsAg positivity was found among these had no formal education (47.4%) and widowed (22.2%). History of multiple sexual practices, nose piercing and history of abortion had statistically significant association with HBsAg sero-positivity ($p < 0.05$). All of the anti- hepatitis C virus antibody positive pregnant women had history of ear piercing, history of abortion, and positive for human immunodeficiency virus.

Conclusion: This study has shown that intermediate and low prevalence of hepatitis B virus and hepatitis C virus infection among pregnant women respectively. Increasing awareness of transmission of HBV through multiple sexual practice and nose piercing is needed. Routine screening of pregnant women for hepatitis B virus and hepatitis C virus is also recommended.

Key Words: *Hepatitis B virus, Hepatitis C virus, pregnant women, Dessie Referral Hospital*

1. Introduction

1.1. Background and Statement of the Problem

Viral hepatitis is an inflammation of the liver due to viral infection. It is a global public health problem affecting millions of people every year. Five different types of hepatitis viruses (A – E) are responsible for viral hepatitis (1, 2). Of these five viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most common types of viruses that infect the liver. These viruses are members of two different viral families, but both display a strong hepatotropism and can cause acute or chronic infections. Those chronically infected with HBV and HCV are at risk of serious illness and death from cirrhosis and hepatocellular carcinoma (HCC) (3).

Hepatitis B virus is an enveloped deoxyribonucleic acid (DNA) virus belonging to the family *Hepadnaviridae*. The viral genome consists of partially double-stranded DNA with a short, single stranded piece. It comprises 3200 nucleotides, making it the smallest DNA virus known (3, 4). The virus is transmitted through exposure to infectious blood, semen, and other body fluids (3). Hepatitis B virus can also be transmitted from infected mothers to infants or from family members to infants in early childhood (5). Transmission may also occur through unsafe sexual intercourse, transfusions of HBV-infected blood and blood products, contaminated injections during medical procedures, and sharing of needles and syringes among injecting drug users (6).

World Health Organization (WHO) estimates that about two billion people have been infected with HBV worldwide and more than 240 million people are chronically infected with the virus. About 500, 000 – 700, 000 people die annually as a result of HBV infection (2). During HBV infection the risk of chronicity varies greatly with the age at which the infection is acquired. For neonates and children younger than one year who acquire the infection, the risk of the infection becoming chronic is 90%. For children aged 1–5 years, the risk is about 30%, and for children older than 5 years and for adults, the risk decreases to around 2% (6).

Hepatitis C virus is a small single-stranded ribonucleic acid (RNA) virus that belongs to the *Flaviviridae* family (3). This virus is mostly transmitted through exposure to infectious blood. This may happen through transfusions of HCV-infected blood and blood products, contaminated injections during medical procedures, and sharing of needles and syringes among injecting drug users (7). Vertical transmission from infected mother to fetus or transmissions through unsafe

sexual contact are other possible modes of transmission of the virus (5). Infection with HCV is a major cause of chronic hepatitis around the world and about 2 – 3% of the world populations are living with HCV infection. More than 350, 000 deaths are attributed to HCV infection each year, most of which are caused by liver cirrhosis and HCC (7).

Hepatitis B virus and HCV are different in carriage rate, natural history and mother to fetus transmission rate (8). For instance about 75 - 85% of patients with HCV will develop chronic infection and about 10 - 15% of patients with HCV develop liver cirrhosis (9). But about 10% of adult patients with HBV will develop chronic infection and about 15 – 40% of those chronically infected patients develop liver cirrhosis and HCC (10). The rate of chronic infection is affected by many factors such as age at time of infection, gender, ethnicity, and the development of jaundice during the acute infection (9). The main reason for the difference in the rate of chronic infection is that HCV evades the immune system more easily than HBV; because HCV mutates more rapidly (8). Hepatitis C virus spreads throughout the immune system by mutating certain parts of its genome; making cluster of differentiation ($CD8^+$) T- cells unable to find them and destroy them. But undergoing this process also disables some of its ability to replicate. Pregnancy causes a woman's immune system to withhold T-cells in order to prevent any attack on the growing fetus. This in turn, allows the virus to replicate without opposition by T-cells. In this case, escape mutations were lost, meaning that the virus didn't need to mutate. This condition is responsible for the developments of virus strains that replicates faster and easily transfer to the fetus (11, 12).

Hepatitis B virus and HCV are also different in their vertical transmission rate. The risk of vertical transmission of HBV depends on the time at which the pregnant woman acquired infection, and on her statuses of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg). The risk of perinatal HBV infection in an infant with an HBsAg positive mother is less than 10% if the mother's HBeAg status is negative. But it is about 70 – 90% if the mother's HBeAg status is positive (5, 13). Similarly the risk of vertical transmission of HCV depends on positivity of the mother for HCV RNA and if the mother is positive for HCV RNA, the rate of vertical transmission is about 4.3% if she is not co-infected with human immunodeficiency virus (HIV). If the mother is co-infected with HIV, the rate of vertical transmission increases up to 19.4% (5). If infected at birth, an infant has approximately a 90% chance of becoming a chronic

HBV carrier. Although rates of new infection and acute disease are highest among adults, the rate of progression from acute to chronic HBV infection is less for adult acquired infection (13).

Viral hepatitis during pregnancy is associated with high risk of maternal complications including premature contractions, preterm delivery, placental separation, premature rupture of membranes, vaginal bleeding, gestational diabetes mellitus and mortality (14, 15). Carriers of HBsAg have increased risk of gestational diabetes mellitus, antepartum haemorrhage, and threatened preterm labour (16). This may be related to the chronic inflammatory state in these subjects. Hepatitis B virus and HCV during pregnancy have a high vertical transmission rate, causing perinatal mortality, congenital malformations and low birth weight (17). Neonatal hepatitis can lead to chronic virus carriage, which in turn may lead to liver cirrhosis and HCC in young adults (5). Infection in the neonatal period is associated with failure to produce antibody to HbsAg, allowing chronic carriage to occur in nearly 100% which is a risk to develop HCC (13).

The prevalence of HBV infection varies greatly in different regions of the world and it is highly endemic in areas such as sub-Saharan Africa, Asia, the Pacific Basin, parts of the Middle East and the Amazon Basin (18). The global burden of HBV infection in pregnant women is not exactly known but the prevalence of infection among women of child-bearing age group is about 8.16% (19).The global prevalence of HCV infection in pregnant women ranges from 0.15 - 2.4% in developed countries and 1 - 8% in developing countries (20). The mother to child transmission of these viruses may occur before birth through transplacental transmission, during delivery, or postnatal transmission during care or through breast milk (13, 20).

Although different studies on the prevalence of HBV and HCV have been conducted in different parts of Ethiopia, most of them focused on investigating the prevalence of HBV and HCV among blood donors (21, 22), HIV infected individuals (23), health care workers (24) and medical waste handlers (25). Only very few studies were conducted in Jimma town (26), Gondar town (27), and Debre-Tabor town (28) to determine the prevalence of HBsAg among pregnant women. To our knowledge, only one study was conducted in Gondar town to determine the prevalence of anti-HCV antibody among pregnant women (27). We believe that, there is very limited or no data about the magnitude of HBV and HCV prevalence among pregnant women in Dessie town and the adjacent administrative areas. Therefore, the aim of this study was to provide baseline information on the magnitude of HBV and HCV infections among pregnant women together

with the associated risk factors in Dessie town. We believe that this study provided relevant and recent information valuable for treatment, prevention and control of HBV and HCV infection.

1.2. Literature Review

The magnitude of HBV and HCV infections among pregnant women was reported at different settings with difference in occurrence. For example, a study conducted in India designed to investigate the sero-prevalence of HBsAg in pregnant women and possible risk factors for perinatal HBV transmission showed a prevalence of 0.9% (29). In the same country the prevalence of anti-HCV antibody was 1.03% (30).

In Italy, the prevalence of chronic HCV infection was evaluated in 15,250 cohorts of pregnant women. The result of this study showed 2.4% of women were positive for anti-HCV antibody and 72% of those anti-HCV antibody positive women were positive for HCV RNA. In this study intravenous drug abuse, history of blood transfusion, accidental blood contact in health care workers and sexual intercourse with HCV positive partner were the major risk factors (31).

A cross sectional study conducted in Pakistan; aimed to assess the frequency of anti – HCV antibody, HBsAg and risk factors in pregnant women in 2006 – 2007 revealed a prevalence of 7% and 4.6% for anti - HCV antibody and HBsAg respectively. Previous history of surgery, multiple injection therapy and blood transfusion were observed as risk factors among anti – HCV antibody and HBsAg positive pregnant women (32).

In 2008, a cross sectional study conducted in Iran showed that the prevalence of HBsAg was 0.7% and that of anti-HCV antibody was 0.2% among pregnant women (33). Another cross-sectional study conducted on pregnant women in Sana'a, Yemen by the year 2011 reported a 10.8% and 8.5% prevalence of HBsAg and anti-HCV antibody respectively. Low parity and educational status below secondary level were significantly associated with anti- HCV sero-positivity (34).

In Saudi Arabia, the prevalence of HBV infection among pregnant women in 2011 was reported 4.1%. This study also indicated that the prevalence of HBV increases in old age. History of hospitalization and jaundices were the main risk factors associated with sero-prevalence of HBV infection (35).

Zeba etal's report by the year 2009 in Burkina Faso determine the prevalence of HCV and co-infection with HIV among pregnant women and found an overall prevalence of HCV infection of 2.14%. The prevalence of HCV was 1.75% and 2.38% for HIV - negative and HIV - positive pregnant women respectively. This study revealed that history of blood transfusion and genital excision were risk factors for HCV positivity in pregnant women (36).

A study report in Yaounde, Cameroon, by the year 2012 showed a prevalence of 7.7% for HBsAg among pregnant women. History of contact with known HBsAg positive individual was independent predictor of sero-prevalence of HBV infection (37).

In Mali, the sero-prevalence of HBsAg among pregnant women was reported 8% by the year 2009. This study also showed that 0.38% of HBsAg positive women were co-infected with HIV. Age, parity, genital excision, birth weight of baby and HIV status were not associated with HBV infection (38).

Different studies conducted in different states of Nigeria showed that the prevalence of HBV infection among pregnant women ranges from 6.67 – 9.5% (39 - 43). A study conducted in Anambra State, Nigeria in 2003 revealed that 8.6% of the women were sero-positive for HIV, 9.3% for HBsAg and 0.7% HIV - HBV co-infection. The prevalence rates of the infections were inversely associated with increase in educational status (39). Similarly in Nasarawa State, Nigeria in 2009, a prevalence of 6.67% HBsAg were reported (40). Furthermore, in 2011 another case control study was conducted in Kano state, Northwest Nigeria and reported prevalence of HBsAg among pregnant and non pregnant women were 7.9 and 7.6%, respectively. The risk factors associated with HBV infection were blood transfusion, ear piercing, tattooing, and abortion among pregnant women (41).

In Nigeria other studies to determine the prevalence of both HBV and HCV in pregnant women were carried out. A hospital based cross sectional study conducted in 2009 in Abuja showed prevalence of 9.5% and 0.5% of HBV and HCV respectively (42). In another cross sectional study conducted in Benin City, Nigeria in 2012 the prevalence of HBV and HCV infections were 2.2% and 0.8% respectively (43).

The prevalence of HCV infection among pregnant women was reported 8.6% for HCV antibody and 6.8% for HCV-RNA in Egypt in 2008. In this study old age, history of blood transfusion, HCV infection of the husband or other household members was significantly associated with

positivity of HCV. The same study showed that out of 53 infants tested at first month, 81% were positive for HCV antibody, but only 13% were positive for HCV-RNA. After 6 months, only 3.8% remained positive for HCV RNA (44).In the same country the prevalence of HBsAg was 1.75% in a study conducted in 2011. Family history of HBV infection, previous intravenous injections, hospital admission, and surgeries were the risk factors for acquiring HBV infection (45).

Sero-prevalence study on HBV and HCV infection among pregnant women in Sudan showed that 5.6% of women were positive for HBsAg and 0.6% of women were positive for anti – HCV antibody. Expected risk factors such as age, parity, gestational age, residence, history of blood transfusion, dental manipulations, tattooing and circumcision did not show association with HBsAg sero-positivity (46).

In Ethiopia, only very few studies are conducted among pregnant women to determine HBV and HCV prevalence and associated risk factors. A cross sectional study conducted in 2003 in Jimma University Specialized Hospital and its four training health centers (Jimma, Asendabo, Agaro and Shebe health centers) showed that the overall prevalence of HBsAg was 3.7%. Higher prevalence of HBsAg (7.3%) was reported among pregnant women who have history of abortion. Even though the overall analysis showed no association between history of caesarian section, dental procedure and tattooing, these risk factors were significant predictors of HBV infection among pregnant women living in Jimma town (26).

Another cross sectional study conducted in 2006 to determine the sero-prevalence of HIV, HBV, HCV and syphilitic infections among antenatal care clinic (ANC) attendees in Gondar town found that the prevalence of HBV and HCV were 7.3% and 1.3% respectively (27). In 2004, 5.3% sero-prevalence of HBV infection among pregnant women attending ANC at Debre-Tabor hospital was also reported (28).

1.3. Significance of the Study

Viral hepatitis remains a major source of morbidity and mortality throughout the world. The occurrence of viral hepatitis during pregnancy is associated with high risk of maternal complications. Additionally, HBV and HCV infected pregnant women are at risk of infecting their babies through vertical transmission. Infection at fetal life and early age predisposes an increased chance of becoming chronic virus carrier. Epidemiological data is essential to estimate the burden and distribution of HBV and HCV among pregnant women. However, there are no published data that showed the magnitude of HBV and HCV infection among pregnant women in Dessie town. Therefore, this study provides baseline and important information about the prevalence of HBV and HCV prevalence among pregnant women which will certainly be valuable for program mangers and health planners so as to initiate the relevant prevention and control mechanisms for these viruses.

This study also provides information about risk factors for acquiring HBV and HCV infection among pregnant women and it can be used as a base line data for further study on the area. Therefore, the aims of this study were to assess the sero-prevalence of HBV and HCV infection and also to determine the possible associated risk factors among pregnant women in Dessie town, Northeast Ethiopia.

2. Objectives

2.1. General Objective

- The aim of this study was to assess the sero-prevalence and associated risk factors of HBV and HCV infections among pregnant women at Dessie referral hospital.

2.2. Specific Objectives

- To determine the sero-prevalence of HBsAg among pregnant women at Dessie referral hospital.
- To determine the sero-prevalence of anti- HCV antibody among pregnant women at Dessie referral hospital.
- To identify the possible risk factors of HBV and HCV infections in pregnant women at Dessie referral hospital.

3. Materials and Methods

3.1. Study Area

The study was conducted at Dessie referral hospital. Dessie referral hospital is found in Dessie town. Dessie town is located in the Northeast part of Ethiopia in the South Wollo administrative zone of the Amhara Region, which is 401 km away from the capital city, Addis Ababa. According to the Central Statistics Agency 2007 report, Dessie has an estimated total population of 151, 094. Dessie referral hospital was established in 1962 and has 17 departments with 300 beds. This referral hospital provides services to the population in the surrounding area of the town and the adjacent regions. The antenatal clinic of the hospital serves about 3000 pregnant women per year. The services delivered by the clinic includes assessment of pre-existing health conditions (screening for anemia, syphilis, HIV), vaccination, nutrition counseling, micronutrient supplementation and early detection of pregnancy related complications.

3.2. Study Design and Period

A cross sectional study was conducted from March 1 to May 30, 2014.

3.3. Population

3.3.1. Source Population

The source population was all pregnant women who have access to attend antenatal follow up at Dessie referral hospital.

3.3.2. Study Population

The study population was all pregnant women who attend antenatal follow up at ANC of Dessie referral hospital during the study period.

3.4. Variables

3.4.1. Dependent Variables

- Sero-prevalence of HBV and HCV

3.4.2. Independent Variables

- Age
- Residence
- Marital status
- Educational status
- Occupation
- History of blood transfusion
- History of surgical procedure
- Ear piercing
- Nose piercing
- History of multiple sexual practice
- History of abortion
- Home delivery by traditional birth attendants
- Parity
- Gravidity
- HIV status

3.5. Sample Size and Sampling Technique

3.5.1. Sample Size Determination

The sample size was determined using a single population proportion formula.

$$n = z^2 p (1-p)/ w^2$$

$$= \frac{3.8416 \times 0.5 \times 0.5}{0.0025}$$

$$n = 385$$

Where:

n – The minimum sample size.

Z – Standard normal distribution value at the 95% CI, which is 1.96.

P – The proportion of sero-positive pregnant women, since there was no previous study in the area we take it as 50%.

W – The margin of error, taken as 5%.

3.5.2. Sampling Technique

Systematic random sampling method was used to select the study participants among ANC attendants. On average, about 12 pregnant women per day were expected to visit ANC at Dessie referral hospital and there are 22 working days in a month. Considering three months study period, 792 pregnant women were expected to visit the ANC during the study period. Therefore, to get 385 sample size, the sampling interval was two. Of the first two subjects, one woman was randomly selected by lottery method, and then every 2nd woman was selected to participate in the study.

3.6. Data Collection

3.6.1. Socio Demographic and Clinical Data

After written consent obtained from the study subjects, information concerning socio-demographic and possible risk factors were collected through face to face interview using a questionnaire. Nurses working at Dessie referral hospital ANC interviewed the study subjects using structured questionnaire on socio-demographic and possible risk factors.

3.6.2. Blood Specimen Collection and Processing

Blood specimen was collected by trained laboratory technologist from each study subject. Five milliliter (5ml) of venous blood was collected with plain tube by strictly following standard operational procedures. Then the sample tube was labeled with the subject's code number and the blood specimen was allowed to clot at room temperature and serum was separated by centrifugation at 5000 revolution per minute (rpm) for 10 minutes. All collected serum specimens were tested for HBsAg and anti-HCV antibody using rapid diagnostic test kits according to the manufacturer's guidelines. Similarly, serum specimens were tested for HIV using rapid test kits based on the current national HIV rapid test algorism of Ethiopia (Annex VII).

3.6.3. Quality Control

Before using the prepared questionnaire for collection of information, it was checked for its completeness and validity to get a reliable and clear data. The questionnaire was originally developed in English and translated to Amharic (local language), then retranslated to English to ensure consistency. To assure the quality of the data, the questionnaire was pretested before the actual work and training were given to data collectors. The pre – test was conducted among pregnant women attending gynecology department of Dessie referral hospital.

After collecting the data, the data were checked for completeness. Positive and negative control samples within the test kits were run to assess the performance of the test kits. Known positive and negative serum samples for HBsAg and anti-HCV antibody confirmed by enzyme linked immunosorbent assay (ELISA) technique was obtained from blood donation center of the

Ethiopian Red Cross Association, Dessie branch. This known serum sample was analyzed before the actual investigation as external quality control of the test kits.

3.7. Data Analysis and Interpretation

After completion of data collection, the data were checked for its completeness. Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 16.0. Descriptive statistics was performed. Association between possible risk factors and sero-positivity of HBV or HCV infection was determined using bivariate and multivariate logistic regression analysis. Odds ratio was used as a measure of strength of association. The 95% confidence interval and P – value less than 0.05 was taken as statistically significant.

3.8. Ethical Consideration

The study was conducted after getting institutional ethical clearance from Research and Ethical Review Committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Official permission from Dessie referral hospital was also obtained. The study subjects were informed about the study process, its purpose and their right to refuse from the study. Refusal from the study will not affect the benefits gained from the clinic. Written informed consent was obtained from each volunteer. Confidentiality were kept for all the data gained in the process by anonymous data collection and restricting access of people other than data collectors to the data. Pregnant women who found positive for HBsAg and/or anti-HCV antibody were linked to ANC physicians for monitoring and further management.

4. Results

4.1. Socio-demographic Characteristics

A total of 385 pregnant women were included in the study. The mean age of the study subjects were 28.2 years with a standard deviation of ± 5.37 years. Majority of the study subjects (64.7%) were in the age group greater than 30 years. The majority of the study subjects (83.6%) were urban dwellers. Three hundred and fifty three (91.7%) of the study subjects were married and 65.5% attend secondary school and above. Gravidity and parity status showed that about 62.1% were pregnant for more than once (multigravidae) and 53.8% have one or more previous delivery (Table 1).

Table 1: Socio-demographic characteristics of pregnant women attending Dessie referral hospital ANC, Northeast Ethiopia, 2014

Variable		N	%
Age (Years)	18 - 30	249	64.7
	31 - 40	136	35.3
Residence	Urban	322	83.6
	Rural	63	16.4
Marital Status	Single	16	4.2
	Married	353	91.7
	Widowed	9	2.3
	Divorced	7	1.8
Educational Status	No formal education	52	13.5
	Primary school	81	21
	Secondary school and Above	252	65.5
Occupation	Employed	135	35.1
	Housewife	201	52.2
	Daily Laborers	17	4.4
	Others	32	8.3
Gravidity	Primigravida	144	37.4
	Multigravida	241	62.6
Parity	Nullipara	178	46.2
	Multipara	207	53.8

4.2. Sero-prevalence of HBV and HCV Infections

Among the 385 pregnant women screened, the sero-prevalence of HBsAg and ant-HCV antibody were 19 (4.9%) and 3 (0.8%) respectively. The highest prevalence of HBsAg 5.9% (8/136) was observed among the age group of greater than 30 years old. Relatively higher prevalence of HBsAg 5.6% (18/ 322) was observed among urban dweller pregnant women. The highest prevalence of HBsAg was observed in widowed women with a prevalence rate of 22.2% (2/9) followed by singles 18.8% (3/16). An inverse relationship between the educational status of the women and the sero-prevalence of HBsAg was observed. The prevalence of HBV infection was found higher (25%) among pregnant women that had no formal education but the prevalence was relatively lower among pregnant women that had educational level of secondary school and above. Moreover, the prevalence of HBV infection was higher, 29.4% (5/17) among pregnant women whom are daily laborers by occupation (Table 2).

The current study also determined the prevalence of HBV infection among HIV positive and HIV negative pregnant women. Accordingly, fifty three pregnant women (13.8%) were positive for HIV. Of these 53 HIV positive pregnant women, 22.6% (12/53) were co-infected by HBV. In the current study there was no HBV and HCV co-infection.

The overall sero-prevalence of HCV was 0.8% (3/385). All the three pregnant women infected by HCV were also co-infected by HIV (Table 2). Regarding gravidity status, all of the three anti-HCV antibody positive pregnant women were pregnant for two and more times. In relation to parity about 66.7% (2/3) anti-HCV antibody positive study subjects have two and above previous delivery.

4.3. Exposure to Risk Factors for HBV and HCV Infections

In this study, exposure to different risk factors for HBsAg and anti-HCV antibody positivity and distribution of HBsAg and anti-HCV antibody was assessed. It was found that 8.6% of the pregnant women had nose piercing, 74.5% ear piercing, 23.6% history of having multiple sexual partners, 14.3% history of abortion, and 10.6% history of home delivery by traditional birth attendants, 1% history of blood transfusion, 3.1% history of surgical procedures. HIV co-infection was also considered as possible risk factor for Both HBV and HCV infection. Accordingly, 13.8% of the pregnant women were also positive for HIV.

Among pregnant women that had a history of abortion, 23.6% (13/55) were positive for HBsAg and the history of abortion was significantly associated with infection caused by the HBV (COR 16.7; 95% CI 6 – 46, P < 0.001). Fifteen of the 91 pregnant women with history of multiple sexual practice, 15 women were positive for HBsAg (Table 2).

In order to investigate the association of socio-demographic variables and other factors with HBsAg positivity, both bivariate and multivariate logistic regression analysis were done. Bivariate logistic regression analysis showed that history of nose piercing (COR 9.9; 95% CI 3.6 – 26.9, P < 0.001) and history of having multiple sexual partners (COR 14.3; 95% CI 4.6 – 44.4, P < 0.001) were significantly associated with HBV infection. Similarly, pregnant women that had history of home delivery by traditional birth attendants (COR 5.7; 95% CI 2.1 – 15.4, P = 0.001) and HIV co-infection (COR 13.6; 95% CI 5 – 36.5, P < 0.001) had a higher risk for sero-prevalence of HBV (Table 2). On the other hand, there was no statistically significant association between age, residence, marital status, occupational status, ear piercing, history of surgical procedures, history of blood transfusion and parity status with that of HBV infection among pregnant women.

Multivariate logistic regression analysis showed that not having formal education, nose piercing, history of multiple sexual practices and history of abortion were retained as determinant factors for HBV infection (P < 0.05). Nevertheless, there was no statistically significant association between gravidity status, home delivery by the help of traditional birth attendants and HIV status with that of HBV infection (Table 2).

Educational status was also found determinant factor for HBV infection in the multivariate model and pregnant women without formal education had a higher risk of acquiring HBV

infection (COR 31.6; 95% CI 4.5 – 225, P = 0.001) than pregnant women that had formal education (Table 2). Pregnant women having history of multiple sexual practices were 13 times (AOR 13.5; 95% CI 2.3 – 78, P = 0.004) more likely of being infected by HBV than pregnant women who had no history of multiple sexual practices. Likewise pregnant women that had history of abortion were at a higher risk of being infected by HBV (AOR 9.1; 95% CI 1.9 – 44, P = 0.005) than pregnant women with no history of abortion.

Table 2: Possible risk factors and their association with HBsAg positivity at Dessie Referral Hospital ANC, Northeast Ethiopia, 2014

Variable		HBsAg Status		COR (95% CI)	P – Value	AOR (95% CI)	P – Value
		Positive n (%)	Negative n (%)				
Age	18 -30	11 (4.4)	238 (95.6)	1		-	-
	31 – 42	8 (5.9)	128 (94.1)	1.35 (0.5 – 3.44	0.527	-	-
Residence	Urban	18 (5.6)	304 (94.4)	1			
	Rural	1(1.6)	62 (98.4)	0.23 (0.03 – 2.08)	0.210	-	-
Marital Status	Single	3 (18.8)	13 (81.2)	1		-	-
	Married	13 (3.7)	340 (96.3)	0.2 (0.4 – 0.6)	0.010	-	-
	Widowed	2 (22.2)	7 (77.8)	1.2 (0.2 – 9.3)	0.835	-	-
	Divorced	1 (14.3)	6 (85.7)	0.72 (0.06 – 8.5)	0.796	-	-
Educational Status							
No Formal Education		13 (25)	39 (75)	27.7 (7.5 – 101)	0.000	31.6 (4.5 – 225)	0.001
Primary school		3 (3.7)	78 (96.3)	3.2 (0.6 – 16)	0.160	0.9 (0.1 – 6.9)	0.915
Secondary School & Above		3 (1.2)	249 (98.8)	1		1	
Occupation	Employed	6 (4.4)	129 (95.6)	1.4 (0.2 – 12.4)	0.739	-	-
	Housewife	7 (3.5)	19 (96.5)	1.12 (0.13 – 9.4)	0.918	-	-
	Daily Laborers	5 (29.4)	12 (70.6)	12.9 (1.3 – 122)	0.026	-	-
	Others	1 (3.1)	31 (96.9)	1			
Ear piercing	Yes	19 (6.6)	268 (93.4)	-	-	-	-
	No	0	98 (100)				
Nose piercing	Yes	8 (24.2)	25 (75.8)	9.9 (3.6 – 26.9)	0.000	18.1 (2.9 – 114)	0.002
	No	11 (3.1)	341 (96.9)	1		1	
History of multiple sexual practices	Yes	15 (16.5)	76 (83.5)	14.3 (4.6 –44.4)	0.000	13.5 (2.3 – 78)	0.004
	No	4 (1.4)	290 (98.6)	1		1	
History of surgical procedure				-	-	-	-
	Yes	0	12 (100)				
	No	19 (5.1)	354 (94.9)				
History of blood transfusion				-	-	-	-
	Yes	0	4 (100)				
	No	19 (5)	362 (95)				
Gravidity	Primigravida	1 (0.7)	143 (99.3)	1		1	
	Multigravidae	18 (7.5)	223 (92.5)	11.5 (1.5– 87)	0.018	1.65 (0.14– 19.2)	0.690
Parity	Nullipara	9 (5.1)	169 (94.9)	1			
	Multipara	10 (4.8)	197(95.2)	1.76 (0.45 – 6.9)	0.415	-	-
History of abortion	Yes	13(23.6)	42 (76.4)	16.7 (6 –46)	0.000	9.1 (1.9 – 44)	0.005
	No	6 (1.8)	324 (98.2)	1		1	
Home delivery	Yes	7 (17.1)	34 (82.9)	5.7 (2.1 – 15.4)	0.001	1.2 (0.24– 6.2)	0.807
	No	12 (3.5)	332 (96.5)	1		1	
HIV status	Positive	12(22.6)	41 ((77.4)	13.6 (5.1 –36.5)	0.000	0.6 (0.12 – 3.68)	0.600
	Negative	7 (2.1)	325 (97.9)	1		1	

COR – Crude odds ratio, AOR – Adjusted odds ratio, CI – Confidence interval

Only three pregnant women were positive for anti-HCV antibody. Due to these small number of HCV positive women, the different categories of the variables do have zero cell values. Therefore, it was impossible to conduct logistic regression analysis. However, all of the three HCV sero-positive pregnant women had history of exposure to risk factors like ear piercing, history of multiple sexual partners, history of abortion, history of home delivery by traditional birth attendants and HIV positive (Table 3).

Table 3: Possible risk factors and their association with ant-HCV antibody positivity at Dessie Referral Hospital ANC, Northeast Ethiopia, 2014

Variable		Total n (%)	HCV anti-body status	
			Positive n (%)	Negative n (%)
Ear piercing	Yes	287 (74.5)	3 (1)	284 (99)
	No	98 (25.5)	0	98 (100)
Nose piercing	Yes	33 (8.6)	1 (3)	32 (97)
	No	352 (91.4)	2 (0.6)	350 (99.4)
History of multiple sexual practices	Yes	91 (23.6)	3 (3.3)	88 (96.7)
	No	294 (76.4)	0	294 (100)
History of surgical procedure	Yes	12 (3.1)	0	12 (100)
	No	373 (96.9)	3 (0.8)	370 (99.2)
History of blood transfusion	Yes	4 (1)	0	4 (100)
	No	381 (99)	3 (0.8)	378 (99.2)
Gravidity	Primigravida	144 (37.4)	0	344 (100)
	Multigravida	341 (62.6)	3 (1.2)	238 (98.8)
History of abortion	Yes	55 (14.3)	3 (5.5)	52 (94.5
	No	330 (85.7)	0	330 ((100)
Parity	Nullipara	178 (46.2)	0	178 (100)
	Multipara	207 (53.8)	3 (1.4)	204 (98.6)
Home delivery	Yes	41 (10.6)	3 (7.3)	38 (92.7)
	No	344 (89.4)	0	344 (100)
HIV status	Positive	53 (13.8)	3 (5.7)	50 (94.3)
	Negative	332 (86.2)	0	332 (100)

5. Discussion

Hepatitis B and Hepatitis C viruses are among the most prevalent infectious agents that causes liver disease in humans worldwide (2, 7). Hepatitis B virus and HCV infections affecting pregnant women may result in severe disease to the mother and chronic infection to the newborn (5, 6). In this study, the overall sero-prevalence of HBsAg and anti- HCV antibody were 4.9% and 0.8% respectively.

The prevalence of HBsAg among pregnant women found in Dessie was nearly similar to the report from Debre-Tabor town which was 5.3% (28). By another study, a 3.7% prevalence of HBV infection among pregnant women was previously reported in the southwestern part of Ethiopia, Jimma (26). Different reports showed different prevalence of HBV infection among pregnant women in different parts of the world. The prevalence of HBV infection among pregnant women was reported 5.6% in Sudan (46), 4.1% in Saudi Arabia (35) and 4.6% in Pakistan (32). On the other hand, lower prevalence of HBV infection was reported in India (0.9%) (29), Iran (0.7%) (33) and Egypt (1.75%) (45). The lower prevalence in these previous studies might be due to differences in diagnostic methods in which the test method were ELISA but rapid test kits were used in the present study. However, higher prevalence of HBsAg were reported in Gondar town, Ethiopia 7.3% (27), Yemen 10.8% (34), Cameroon 7.7% (37), Mali 8.0% (38) and Nigeria 9.3% (40). These discrepancies in the prevalence of HBsAg might be due to the difference in sample size, awareness of the routes of HBV transmission, the differences in demographic characteristics of the study population such as traditional practices and sexual practices.

The prevalence of HBV and HCV infections found in the current study can be graded as intermediate and low prevalence respectively according to WHO criteria (47, 48). The prevalence of HBV infection can be divided into three main categories namely: high when the prevalence is > 8%, intermediate when the prevalence is between 2-8% and low when < 2% (47). Hepatitis C virus infection can be graded high, moderate or low when the prevalence is > 3.5%, 1.5% - 3.5% and < 1.5% respectively (48).

In this study the prevalence of anti-HCV antibody was 0.8%. This finding is in line with previous reports among pregnant women in Sudan (0.6%) (46) and Benin City, Nigeria (0.8%) (43). Relatively similar HCV prevalence was observed in India (1.03%) (30), Iran (0.2%) (33) and

Abuja, Nigeria (0.5%) (42). In contrast, higher prevalence of anti-HCV antibody was reported from Italy (2.4%) (31), Pakistan (7.0%) (32), Yemen (8.5%) (34), Burkina Faso (2.14) (36), Egypt (8.6%) (45) and Gondar, Ethiopia (1.3%) (27). This disparity of the sero-prevalence of HCV might be due to the differences in diagnostic methods, the difference in sample size, the difference in hepatitis epidemiology in these countries, awareness about risk factors, socio-cultural and behavioral differences.

Even though the difference was not statistically significant, in the present study the prevalence of HBsAg was higher in the age group greater than 30 years old (5.9%) as compared to younger age group women (≤ 30 years old) (4.4%). This finding is in agreement with the results which were reported in previous studies conducted in Iran (33) and Saudi Arabia (35). Similarly, in a study conducted in Jimma, Ethiopia the age group between 30 – 34 years and greater than 40 years old had higher prevalence of HBV infection (26). On the contrary, other studies conducted in Debre-Tabor town of Ethiopia (28) and Kano state of Nigeria (41) reported higher HBsAg prevalence among the age group less than 30 years old. This discrepancy might be due to variation in the number of study population in each age group.

Regarding anti-HCV antibody positivity, all positive pregnant women were in the age group less than 30 years old. This finding is in line with similar study conducted in India in which majority of anti-HCV antibody positive cases was observed in the age group less than 30 years old (30). In a study conducted in Benin city of Nigeria a dominant proportion of HCV prevalence was observed among the age group less than 32 years old (43).

In the current study, with the exception of educational status, all the other socio-demographic variables didn't show significant association with HBV infection among pregnant women. Similar associations of demographic factors were also reported in Cameroon (37). In the present study, prevalence of HBV infection was found inversely associated with increased educational status. Pregnant women who had no formal education had a higher risk of infection than pregnant women with educational level of secondary school and above. This finding is in line with studies conducted in Nigeria among the same study population (39, 40). This higher prevalence of HBV among pregnant women who had no formal education might be due to the lack of awareness about the route of transmission and methods of prevention.

History of nose piercing, history of multiple sexual partners and history of abortion were found to be significant predictors of HBV sero-prevalence among pregnant women in the present study. There was also other report that documented similar findings in Kano state of Nigeria (41). In addition, history of abortion was significantly associated with HBV infection in a study conducted in Jimma town (26). In the present study, having history of abortion increased the risk of having HBV infection more than nine times as compared with those who had not suffered such experience. On the other hand, though multivariate logistic analysis showed no significant association, bivariate logistic regression analysis indicated that history of home delivery by traditional birth attendants, multigravida and HIV infection were found significant predictors of HBV infection in the present study.

In this study history of blood transfusion and surgical procedures were not associated with the sero-prevalence of HBV. In contrast to this finding, history of surgical procedures and history of blood transfusion have been significantly associated with sero-prevalence of HBV prevalence in studies conducted in Pakistan (32), Kano State of Nigeria (41) and Egypt (45). In the present study, we noticed that the number of pregnant women that had history of blood transfusion or history of previous surgery was small which might contributed for the absence of these variables association with HBV infection among pregnant women. Transfusion-transmissible infectious agents such as HBV, HCV, HIV and syphilis are among the greatest threats to blood safety for transfusion recipients and pose a serious public health problem (49).

Higher prevalence of anti-HCV antibody were observed among study subjects who had history of ear piercing, history of having multiple sexual partner, multi-gravida, history of abortion, multi-parity and home delivery by traditional birth attendants. This finding is in agreement with studies reported from Sudan (46) and Benin City of Nigeria (43). Previous surgical procedure, history of frequent injections and ear piercing were reported as risk factors for HCV infection in a study conducted in Egypt (50).

The current study revealed that there was no significant association between sero-prevalence of HBsAg and HIV status of the women in multivariate logistic regression analysis ($P > 0.05$). The reason for this might be due to the presence of small number of HIV positive pregnant women as compared to HIV negative pregnant women. A similar picture was observed in previous studies

conducted in Mali (38) and Benin City of Nigeria (43) in which HIV status of pregnant women did not significantly affect the prevalence of HBV infection.

In the present study, there was no HBV and HCV co-infection. This is supported by a study conducted previously among blood donors in Amhara and Tigray national states where there was no HBV and HCV co-infection (22).

6. Limitation of the Study

- Only HBsAg were used for the detection of HBV infection. Other sero-markers were not included due to budget constraints.
- Only rapid test kits were used for the detection of HBsAg and anti-HCV antibody. Usually confirmatory test is commonly used to evaluate positive rapid test results. Due to resource constraints this study was unable to conduct confirmatory tests for those positive results.
- Since this study was hospital based study, it may not reflect the true prevalence among the general population in the area.

7. Conclusion and Recommendation

7.1. Conclusion

This study has found that the sero-prevalence of HBsAg and ant-HCV antibody among pregnant women was 4.9% and 0.8% respectively. Results from this study have shown that HBV prevalence in pregnant women is of intermediate prevalence and multiple sexual practice, nose piercing and history of abortion were associated with HBV infection in the area.

The study also showed a low prevalence of HCV infection in the area. Majority of anti-HCV antibody positive pregnant women had history of exposure to risk factors like ear piercing, history of multiple sexual partners, history of abortion, history of home delivery by traditional birth attendants and HIV positive

7.2. Recommendation

- Free screening of all pregnant women for HBV and HCV should be made as part of routine antenatal care service.
- Awareness creation should be made for pregnant women on risk factors such as multiple sexual practice and nose piercing for the transmission of HBV.
- Further study on HBsAg and anti-HCV antibody positive pregnant women should be conducted to determine HBeAg and HCV – RNA positivity which is important to know the extent of vertical transmission in the area.
- Further population based study should be conducted to know the exact prevalence of HBV among the general population in the area.

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Annex I: English Version Information Sheet

University of Gondar

College of Medicine and Health Sciences

School of Biomedical and Laboratory Science

Study Title: Sero-prevalence and associated risk factors of HCV and HBV infections among pregnant women attending ANC at Dessie Referral Hospital; Northeast Ethiopia.

Purpose

I planned to conduct a study with objective of investigating the magnitude of HBV and HCV infection in pregnant women. The knowledge gained from this work is believed to help the management and control of these viruses.

Participation

I am asking you and others to voluntarily participate in this study. What is expected from everyone is to be examined for HBV and HCV and be asked to answer few questions in relation to risk factors. The laboratory examination involves collection of 5 ml venous blood. All samples are collected using sterile and disposable equipments: tubes, syringes and needles.

Risks and Discomforts Associated

Taking 5ml of blood doesn't have any harm to your health except minor needle brick injury pain which lasts only for micro second. However if you have any discomfort you will be seen by physician.

Benefits

If there is any positive finding in laboratory investigation the result will be communicated to your physician and prescription of treatment and advice will be effected.

Confidentiality

Any information that I collect about you during this research will be kept confidential.

Information about your identity will be put away after recording your file; and kept in a secured place. Only the principal investigator will be able to link your identity with the code number.

Sharing the Result

At the end of this study I will write a report about the results of the study through publication or any other means. The reports won't bear any information relevant to your personality e.g. your name or identity. I assure you the confidentiality of such information. Thus I also need your permission to use the test results for writing a report.

Right to Refuse

Since participation in this study is entirely voluntary. You can refuse to participate in this research at any time. Your refusal to participate in this study will not affect any of the benefits you are supposed to get from the clinic.

Contact Address

If you have any further question and in case of urgency you can contact the principal investigator at any time using the following address:-

Name:- Mohammed Seid (Principal investigator)

Address: - University of Gondar College of Medicine and Health Sciences School of Biomedical and Laboratory Science

City:- Gondar

Telephone (mobile):- +251920272420

Email:- mohammed.seid@yahoo.com

Annex II: English Version Consent Form

I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind.

I……do her by give consent to ……. to include me in this study. I have been given the necessary information about the study. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal has been explained to me in the language I understand.

Name of the study participant: _____

Participant's Signature: _____

Name and role of the person obtaining consent: _____

Signature of person obtaining consent: _____

Date: _____

Annex III: Amharic Version Information Sheet

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የባዮሜድካልና ላቦራቶሪ ሣይንስ ትምህርት ቤት

ለጥናቱ መረጃና ተሳታፊነት መግለጫ ቅጽ

የጥናቱ ርዕስ፡ ሄፓታይተስ ቢ እና ሲ የተባሉ ጉበት አምጪ ቫይረሶች በነፍሰ ጡር እናቶች መካከል ያላቸዉ ስርጭትና አጋላጪ ሁኔታዎች፡፡

የጥናቱ ዓላማ

የሄፓታይተስ ቢ እና ሲ ቫይረሶች በነፍሰ ጡር እናቶች መካከል ያለዉን ስርጭት ለማጥናት የታቀደ ነዉ፡፡

በጥናቱ ስለመሳተፍ

በዚህ ጥናት መሳተፍ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ ስለሆነም በጥናቱ እንዲሳተፉ ፈቃደኛነትዎን እንጠይቃለን ፡፡ ለመሳተፍ ከፈቀዱ 5 ሚሊ ሊትር የደም ናሙና ከክንድዎ ተወስዶ የላቦራቶሪ ምርመራ ይደረግሎታል ፡፡ የላቦራቶሪ ምርመራውም ሄፓታይተስ “ቢ” እና “ሲ” ቫይረስን በደሞ ዉስጥ መኖር ና አለመኖር ማረጋገጥ ይሆናል ፡፡ የደም ናሙናዉም የሚወሰደዉ ንጽህናዉ በተጠበቀ አዲስ እና በታሸገ መርፌ ና ስሪንጅ ነዉ፡፡

በጥናቱ ሊከሰቱ የሚችሉ ተያያዥ ችግሮች

አምስት ሚሊ ሊትር የደም ናሙናዉን ለመዉሰድ መርፌ ሲገባ ከሚፈጥረዉ የቅጽበት የህመም ስሜት በስተቀር የጎላ ችግር አያመጣም ነገር ግን ምቾት ካልተሰማዎት ሀኪም እንዲያይዎት ይደረጋል ፡፡

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

የደም ናሙና የላብራቶሪ ዉጤት ምንም አይነት ችግር ካሳየ የመድሃኒት ትእዛዝ ና የባለሙያ ምክር ይሰጥዎታል፡፡

የጥናቱ መረጃዎች ሚስጥራዊነት

በጥናቱ ውስጥ የተሰበሰቡ ማናቸዉም ግላዊ መረጃዎች ሚስጥራዊነታቸዉ የተጠበቀ ይሆናል፡፡ ከማንነትዎ ጋር በቀጥታ ተያያዥነት ያላቸዉ መረጃዎች በሙሉ በዋና ተመራማሪዉ ሚስጥራዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኃላ ብቻ ለምርምር ሂደቱ የሚዉሉ ይሆናሉ፡፡

የጥናቱን ዉጤት ስለማሳወቅ

የዚህ ጥናት ዉጤት በተለያዩ የህትመት ውጤቶች የሚቀርብ ሲሆን ይህ ከማንነትዎ ጋር የተያያዘ ምንም አይነት መረጃን አያካትትም፡፡ ስለዚህም የጥናቱን ዉጤት በሪፖርት እናቀር በዉ ዘንድ ፈቃድዎን እንጠይቃለን፡፡

ከጥናቱ ስለ መዉጣትና ስለማቋረጥ

ይህ ጥናት በፈቀደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማንኛዉም ወቅት በፈቃድዎ ከጥናቱ መዉጣት ይችላሉ፡፡ ከጥናቱ ቢወጡም እንኳን የተለመደዉን የህክምና እርዳታ በጤና ተቋሙ ዉስጥ በማንኛዉም ጊዜ የማግኘት መብት አልዎት፡፡

ከጥናቱ ጋር በተያያዘ ማናቸውም ጥያቄ ቢኖርዎ በሚከተለዉ አድራሻ ጥያቄዎን ማቅረብ ይችላሉ፡-

ዋና ተመራማሪ፡- መሀመድ ሠይድ

አድራሻ፡- የጎንደር ዩኒቨርስቲ የህክምናና ጤና ሣይንስ ኮሌጅ የባዮሜድካልና ላቦራቶሪ ሣይንስ ትምህርት ቤት

ሞባይል ስልክ፡- +251920272420

ኢሜል፡- mohammed.seid@yahoo.com

Annex IV: Amharic Version Consent Form

ስለ ስምምነቱ ማረጋገጫ ፊርማ

እኔ ስሜ ከታች የተገለፀዉ የጥናቱ ተሳታፊ ለመሆን ስወስን የጥናቱን አላማዎች አሰራሮችና ቅድመ ሁኔታዎች በግልጽ በመረዳትና ከጥናቱ ተሳታፊነት ፈቃደኛነቴን በማንኛዉም ደረጃ የማንሳት መብቴን በማረጋጥ ነዉ፡፡

እኔ ---------- በጥናቱ ተሳታፊ መሆኔን በፊርማዬ እያረጋገጥሁ ይህንን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎች በሚገባ የተረዳሁ ና ከጥናቱ በማንኛዉም ደረጃ እራሴን ለመሰረዝ ብወስን ተገቢ የሆኑ ህክምናዎች ና እገዛዎች ሁሉ እነደማይነፍጉኝ በማመን ነዉ፡፡ እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ ፡፡

የጥናቱ ተሣታፊ ሙሉ ስም፡ ----------

ፊርማ፡ ----------

የተመራማሪዉ ሙሉ ስም፤ ----------

ፊርማ፡ ----------

Annex V: English Version Questionnaire

University of Gondar

College of Medicine and Health Sciences

School of Biomedical and Laboratory Science

Questionnaire to assess socio-demographic characteristics and Risk factors

For data collectors: Please write on the space provided or circle on the answer among choices accordingly.

	Code : _____	Date: _____	
Section One: Background of the study participants			
1	How old are you?	[_____] age in years	
2	Current residence?	1. Urban 2. Rural	
3	Marital Status	1. Single 2. Married 3. Widowed 4. Divorced	
4	Educational Status	1. No formal education 2. Primary school (1 – 8) 3. High school and Above	
5	Occupation	1. Government employed 2. Private employed 3. Housewife 4. Daily laborer 5. Merchant 6. Farmer 7. Others (Specify) _____	

Section Two: Possible risk factors			
6	Ear piercing?	1. Yes 2. No	
7	Nose piercing?	1. Yes 2. No	
8	History of multiple sexual practices?	1. Yes 2. No	
9	History of surgical procedure?	1. Yes 2. No	
10	History of blood transfusion?	1. Yes 2. No	
11	How many pregnancies do you have including this one?	1. First time 2. Second time 3. Three and above (Specify in numbers) _____	
12	Do you have abortion /stillbirth/ history?	1. Yes 2. No	
13	How many deliveries do you have previously?	1. No previous delivery 2. One 3. Two and above (Specify in numbers) _____	
14	Home delivery by traditional birth attendants?	1. Yes 2. No	
15	HIV status	1. Positive 2. Negative 3. Unknown	

Annex VI: Amharic Version Questionnaire

የጎንደር ዩኒቨርስቲ

የህክምናና ጤና ሣይንስ ኮሌጅ

የባዮሜድካልና ላቦራቶሪ ሣይንስ ትምህርት ቤት

ማህበራዊ ነክ መረጃዎችንና አጋላጭ የሆኑ ሁኔታዎችን ለመዳሰስ የተዘጋጀ መጠይቅ

ለመረጃ ሰብሳቢዎች፤ ጥያቄዉን ከጠየቃችሁ በኃላ መልሱን በተሰጠዉ ክፍት ቦታ ወይም ከተሰጡት አማራጮች አንዱን በማክበብ ይጻፉ፡፡

	ኮድ : _____	ቀን: _____	
ክፍል 1፡ አጠቃላይ ማህበራዊ ነክ መረጃዎች			
1	እድሜ	[_____] ዓመት	
2	የመኖሪያ አካባቢ?	1. ከተማ 2. ገጠር	
3	የጋብቻ ሁኔታ	1. ያላገባች 2. ያገባች 3. ባል የሞተባት 4. የፈታች	
4	የትምህርት ደረጃ	1. መደበኛ ትምህርት ያልተማረች 2. የመጀመሪያ ደረጃ ት/ት (1 — 8) 3. የሁለተኛ ደረጃ ት/ትና ከዚያ በላይ	
5	የሥራ ሁኔታ	1. የመንግስት ተቀጣሪ 2. የግል ድርጅት ተቀጣሪ 3. የቤት እመቤት 4. የቀን ሰራተኛ 5. ነጋዴ 6. ገበሬ	

		7. ሌላ (ይጠቀስ) ___	
ክፍል 2፡ ለሄፓታይትስ ቢ ና ሲ አጋላጭ የሆኑ ሁኔታዎችን የሚዳስሱ ጥያቄዎች			
6	ጆሮዎን ተበስተዉ ያዉቃሉ?	1. አዎ 2. አይደለም	
7	አፍንጫዎን ተበስተዉ ያዉቃሉ?	1. አዎ 2. አይደለም	
8	ከአንድ በላይ የፍቅር ጉደኛ ነበረዎት? (ከአንድ ሠዉ በላይ የግብረስጋ ግንኙነት ነበረዎት?)	1. አዎ 2. አይደለም	
9	ማንኛዉንም አይነት ቀዶ ጥገና አሰርተዉ ያዉቃሉ?	1. አዎ 2. አይደለም	
10	ደም ከሌላ ሠዉ ተሰጥቶዎት ያዉቃል?	1. አዎ 2. አይደለም	
11	የአሁኑን ጨምሮ ለስንተኛ ጊዜ አርግዘዋል?	1. ለመጀመሪያ ጊዜ 2. ለሁለተኛ ጊዜ 3. ለሦስትና ከዚያ በላይ (በቁጥር ይጠቀስ) _____	
12	አስዎርዶዎት ያዉቃል?	1. አዎ 2. አይደለም	
13	ምን ያህል ልጆች ወልደሻል?	1. ወልጄ አላዉቅም 2. አንድ 3. ሁለትና ከዚያ በላይ (በቁጥር ይጠቀስ) ____	
14	በቤት ዉስጥ በልምድ አዋላጅ ተዋልደዉ ያዉቃሉ?	1. አዎ 2. አይደለም	
15	የ “ኤች አይ ቪ” ቫይረስ በደመዎ ዉስጥ አለ? (የሴትዋን ካርድ በማየት የሚሞላ)	1. አዎ (ፖዝቲቭ) 2. አይደለም (ኔጋቲቭ) 3. አይታወቅም	

Annex VII: Laboratory Procedures and Data Collection Format

Serological Test Principle and Procedure for HBsAg

EUGENE^{Đ} HBsAg

The EUGENE^{Đ} HBsAg rapid test (Shanghai Eugene Biotech co., Ltd) is a lateral flow immunochromatorgraphic assay for the qualitative determination of HBsAg (a marker of HBV infection) in human serum or plasma.

Test Principle

The EUGENE^{Đ} HBsAg rapid test a rapid qualitative immunochromatorgraphic assay employing a unique combination of monoclonal dye-conjugate (colloidal gold) and polyclonal solid phase antibodies to selectively identify HBsAg of HBV infection with a high degree of sensitivity. In this test, plasma or serum specimen is added directly to the sample pad. As the test sample flows through the sample pad, the labeled antibody-dye conjugate binds to HBsAg forming an antibody-antigen complex. The pad is in contact with a chromatographic test strip which contains a region of immobilized polyclonal anti-HBsAg antibody in the test line. The antibody-antigen complex moves by capillary action along the strip forming a line of immobilized complex by the zone of antibody in the test line, indicating the presence of HBsAg in the sample (pink line). If no antigen is present, the test line will remain clear. The appearance of a pink line in the control line shows that the test has been carried out correctly.

Test Procedure

1. Bring the complete kit and sample to be tested to room temperature (15 – 30^{O}C) prior to testing.
2. Remove the test device from its protective pouch, lay it on a dry and clean flat surface, and label the device with patient or control number.

3. Use the dropper or pipette to withdraw serum specimen from the specimen collection container and dispense 2 – 3 drops (approximately 80 - 120μl) in to the sample well and start the timer.
4. Wait for colored bands to appear. For strong positive, results may be observed within one minute. For 5ng/ml, read within 5 – 10 minutes; for 1.5 – 2ng/ml read within 15 – 20 minutes. However, to confirm negative results, the complete reaction time 20 – 30 minutes is required. Do not interpret result after 30 minutes.

Interpretation of Results

Negative: One pink line appears in control line, showing the test has been carried out correctly. There will be no line in test region.

Positive: In addition to a pink colored control line, a distinct pink colored band will also appear in the test region. Any shade of color in the test region (T) should be considered positive.

Invalid: A total absence of color in both regions is an indication of procedure error and/or that the test reagent has deteriorated. Repeat the testing using a new device.

Serological Test Principle and Procedure for Anti-HCV Rapid Test

The EUGENE Anti-HCV rapid test (Shanghai Eugene Biotech co., Ltd) is a sandwich lateral flow immunochromatorgraphic assay for the qualitative detection of antibodies to HCV in human serum or plasma.

Test Principle

The EUGENE Anti-HCV rapid test is a lateral flow immunochromatorgraphic assay screening serum or plasma using recombinant HCV proteins. Recombinant antigens of HCV labeled by gold conjugates are used in test band as capture materials, and anti-rabbit HCV antibody is used in the control band. When a sample is added in to the sample well of the device, it migrates through the membrane strip. If the antibodies to HCV present in the specimen, a complex of antibody- gold conjugated recombinant antigens will be formed, which is then captured by

antigen immobilized in the test zone of the membrane, producing a visible pink color band of immunocomplex conjugate on the membrane. The color intensity will depend on the concentration of the ant-HCV present in the sample. Absence of the test band suggests a negative result. The test contains an internal control (C band) which should exhibit a pink colored band of the immunocomplex conjugate regardless of color development on the test band. Otherwise, the test result is invalid and the specimen must be retested with another device.

Test Procedure

1. Bring the complete kit and sample to be tested to room temperature (15 – 30^{O}C) prior to testing.
2. Remove the test device from its protective pouch, lay it on a dry and clean flat surface, and label the device with patient or control number.
3. Hold the dropper vertically and transfer only one drop (about 25µl) of serum or plasma to the specimen well (S) of the test device, then add two drops (about 100µl) of buffer and start the timer.
4. Wait for the red line (s) to appear. The result should be read at 10 – 20 minutes. Do not interpret result after 20 minutes.

Interpretation of Results

Negative: If only the C band is developed, the test indicates that no detectable antibodies to HCV present in the specimen. The result is negative.

Positive: If both C and T bands are developed, the test indicates for the presence of antibodies to HCV in the specimen. The result is positive. Any shade of color in the test region (T) should be considered positive.

Invalid: If no C band is developed, the assay is in valid regardless of color development on the T band. Repeat the assay using a new device.

Serological Test Principle and Procedure for HIV - 1 and HIV – 2

HIV 1/2 STAT-PAK™ (Chembio Diagnostics)

The Chembio HIV 1/2 STAT-PAK™ Assay (CHEMBIO DIAGNOSTIC SYSTEMS, INC., MEDFORD, NY, USA) is a single-use immunochromatographic test for the detection of antibodies to HIV-1 and HIV-2 in whole blood, serum or plasma specimens. The Chembio HIV 1/2 STAT-PAK™ assay is intended for use as a point of- care test to aid in the diagnosis of infection with HIV-1 and HIV-2. This test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results.

Test Principle

The Chembio HIV 1/2 STAT-PAK™ Assay employs a unique combination of a specific antibody binding protein which is conjugated to colloidal gold dye particles and HIV-1/2 antigens which are bound to the solid phase membrane. Whole blood, serum or plasma is applied to the SAMPLE (S) well of test device followed by the addition of running buffer. The buffer facilitates the lateral flow of the specimen and test reagents and promotes the binding of the antibodies to the antigen. The specimen/buffer mixture migrates along the test strip by capillary action, reconstituting the conjugate. If present, the antibodies bind to the colloidal gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST (T) area producing a pink/purple line. In the absence of HIV-1 and HIV-2 antibodies, there is no pink/purple line in the TEST (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device.

Test Procedure

If the specimen to be tested is refrigerated, remove it from the refrigerator and allow it to come to a temperature of 18 to 30°C: (64 to 86°F) prior to testing.

1. Remove the Chembio HIV 1/2 STAT-PAK™ test device from its pouch and place it on a flat surface.
2. Label the test device with patient name or identification number.

3. Touch the 5 µl sample loop provided to the specimen, allowing the opening of the loop to fill with the liquid.
4. Holding the sample loop vertically, touch it to the sample pad in the center of the SAMPLE (S) well of the device to dispense ~5 µL of sample (serum, plasma or whole blood) onto the sample pad.
5. Invert the Running Buffer bottle and hold it vertically (not at an angle) over the sample well. Add 3 drops (~ 105 µL) of buffer slowly, drop-wise, into the SAMPLE (S) well.
6. Read the test result between 15 and 20 minutes after the addition of the running buffer. Reactive test results may be observed and read earlier than 15 minutes. To verify a non-reactive test result, wait the entire 15 minutes after starting the test. Do not read results after 20 minutes.

Interpretation of Test Results

NON-REACTIVE: One pink/purple line in the control (C) area, with no line in the test (T) area indicates a non-reactive Test Result. A non-reactive test result means that HIV-1 and HIV-2 antibodies were not detected in the specimen. The test result is interpreted as negative for HIV-1 and HIV-2 antibodies. However, this does not exclude possible infection with HIV.

REACTIVE: Two pink/purple lines, one in the test (T) area and one in the control (C) area indicate a reactive test result. The line in the test (T) area may look different from the line in the control (C) area. Intensities of the Test and Control Lines may vary. Test result with visible lines in both test (T) and control (C) areas, regardless of intensity, is considered reactive. A reactive test result means that HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The Test Result is interpreted as Preliminary positive for HIV-1 and/or HIV-2 antibodies.

INVALID: A pink/purple line should always appear in the control (C) area, whether or not a line appears in the test (T) area. If there is no distinct pink/purple line visible in the control (C) area, then the test is invalid. Any lines that appear outside of the control (C) area or test (T) area is an invalid test. An invalid test cannot be interpreted. It is recommended that the Invalid test be repeated with a new device.

KHB Rapid test

The rapid test for HIV 1/2 **(**KHB Shanghai Kehua Bio-engineering Co. Ltd, China)is an in-vitro, visually read test for the qualitative determination of antibodies to HIV – 1 and HIV – 2 in human serum, plasma or whole blood. The test is intended to be used as an aid to detect antibodies to HIV 1/2 from infected individuals.

Test Principle

The rapid test of HIV 1/2 from KHB adopts the solid phase colloidal gold immunochromatographic technology for the qualitative detection of antibodies to HIV 1/2. The gold – gp160 conjugate and the gold – gp36 conjugate are coated to the conjugate pad in advance. The test line (HIV type I + II antigens) and the control line (monoclonal antibody against gp160) are pre-coated on the surface of the NC membrane. When the sample that added to the sample pad migrates through the conjugate pad, it reconstitutes and mixes with the colloidal gold - antigen conjugates. The mixture continues to migrate through the NC membrane to the pre-coated antigens or antibody that present on the membrane. A reddish - purple test line will be visible in the strip if there are enough antibodies to HIV 1/2 in the sample. If antibodies to HIV 1/2 are absent, or are present at very low level, then no color will appear in the test line. The control line of reddish – purple is for quality control only and thus does not affect the test result.

Test procedure

1. Remove the KHB device from its packaging, and label it using a code number or a client identification number. Place in a flat surface.
2. Add about 40µl of sample to the sample area and then add one drop of sample diluents to the same area.
3. Read the test result between 15 -30 minutes after the addition of the running buffer. Reactive test results may be observed within 2 - 3 minutes. To verify a non-reactive test result, wait the entire 30 minutes after starting the test.

Interpretation of Test Results

Negative: One pink-purple line in the control (C) area, with no line in the test (T) area indicates a non-reactive test result.

Positive: Two pink-purple lines, one in the test (T) area and one in the control (C) area indicate a reactive test result. The line in the test (T) area may look different from the line in the control (C) area. Intensities of the Test and Control lines may vary. Test result with visible lines in both test (T) and control (C) areas, regardless of intensity, is considered reactive.

Invalid: If there is no distinct pink/purple line visible in the control (C) area, then the test is invalid.

Uni-Gold HIV 1/2 Rapid Test Kit

Uni-Gold™ Recombigen® (Trinity Biotech Plc Bray, Co. Wicklow, Ireland) HIV-1/2 is a single use rapid immunoassay, for the qualitative detection of antibodies to HIV-1 and/or HIV-2 in serum, plasma and whole blood. Uni-Gold™ Recombigen® HIV-1/2 is intended for use in point of care settings as an aid in diagnosis of infection with HIV-1 and/or HIV-2. This test is suitable for use in appropriate multi-test algorithms designed for the statistical validation of rapid HIV test results.

Test principle

Uni-Gold™ Recombigen® HIV-1/2 was designed as a rapid immunoassay and is intended to detect antibodies to HIV-1 and/or HIV-2 in human serum, plasma and whole blood. Uni-Gold™ Recombigen® HIV-1/2 uses proteins representing regions of the HIV virus. If antibodies to HIV-1 and/or HIV-2 are present in the sample, they combine with these proteins and a color reagent and this complex binds to the proteins in the test forming a visible pink/red band in the test region of the device adjacent to the word ‘Test’. The control line should always appear as a visible pink/red band in the control region of the device to indicate that the test device is functioning correctly. A reactive result is indicated by a pink/red band in the test region of the device. A non-reactive result occurs in the absence of detectable levels of antibodies to HIV-1 and/or HIV-2 in the specimen; consequently no visually detectable band develops in the test region of the device.

Test Procedure

1. Allow the kit (unopened devices and wash solution) to reach room temperature (15 – 27°C if previously stored in the refrigerator.
2. Remove the required number of Uni-Gold HIV-1/2 devices from their pouches and lay the devices on a clean flat surface.
3. Label each device with the appropriate patient information / Identification number/.
4. Draw up adequate sample to the first gradation on the Pipette using one of the disposable pipettes included in the kit. Use only the disposable pipette included in the kit and do not reuse. If kit Controls are being run, these must be used as described in the package insert provided with the kit controls.
5. Holding the Disposable Pipette vertically over the sample port, add one free falling drop of sample carefully. Do not add the full volume contained within the disposable pipette allow the sample to absorb into the paper in the sample port. Ensure air bubbles are not introduced into the sample port. Discard the disposable pipette in a biohazard waste container.
6. Holding the dropper bottle of wash solution in a vertical position, add four drops of wash solution to the sample port.
7. Set the timer for 10 minutes and start timing the test.
8. Read test results after 10 minutes but not more than 12 minutes incubation time.

Interpretation of Test Results

Reactive: A pink/red line of any intensity appears in the device window adjacent to word "Test" and a second pink/red line of any intensity appears adjacent to word "Control". This indicates a reactive result that is interpreted as preliminary positive for HIV-1 and/or HIV-2 antibodies.

Non-Reactive: A pink/red line of any intensity appears in the device window adjacent to word "Control", but no pink/red line appears in the device window adjacent to "Test". This indicates a Non-Reactive result that is interpreted as Negative for HIV-1 and/or HIV-2 antibodies.

Invalid: No pink/red line appears in the device window adjacent to word "Control" whether or not a pink/red line appears in the device window adjacent to word "Test". This is an Invalid result that cannot be interpreted. The test should be repeated in duplicate with fresh devices.

Laboratory Data Collection Format

Code No.	Laboratory result for							
	HBsAg				Anti-HCV Ab			
001.	Positive		Negative		Positive		Negative	
002.	Positive		Negative		Positive		Negative	
003.	Positive		Negative		Positive		Negative	
004.	Positive		Negative		Positive		Negative	
005.	Positive		Negative		Positive		Negative	
006.	Positive		Negative		Positive		Negative	
007.	Positive		Negative		Positive		Negative	
008.	Positive		Negative		Positive		Negative	
009.	Positive		Negative		Positive		Negative	
010.	Positive		Negative		Positive		Negative	
011.	Positive		Negative		Positive		Negative	
012.	Positive		Negative		Positive		Negative	
013.	Positive		Negative		Positive		Negative	
014.	Positive		Negative		Positive		Negative	
015.	Positive		Negative		Positive		Negative	
016.	Positive		Negative		Positive		Negative	
017.	Positive		Negative		Positive		Negative	
018.	Positive		Negative		Positive		Negative	
019.	Positive		Negative		Positive		Negative	
020.	Positive		Negative		Positive		Negative	
021.	Positive		Negative		Positive		Negative	
022.	Positive		Negative		Positive		Negative	
023.	Positive		Negative		Positive		Negative	
024.	Positive		Negative		Positive		Negative	

Make an "X" mark for the results